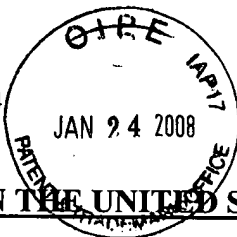


Appl. No. 10/251,364



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : 10/251,364
Applicant : Rapp et al
Filed : September 18, 2002
Title : Production of a Transgenic Avian
By Cytoplasmic Injection

TC/A.U. : 1632
Examiner : Michael C Wilson

Docket No. : AVI-034

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop: RCE, Commissioner for Patents, P.O Box 1450, Alexandria VA 22313-1450 or facsimile transmitted to the U.S. Patent and Trademark Office on or before:

Date June 7, 2006
Signature [Signature]
Name Kyle Yesland

Honorable Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF MARKLEY C. LEAVITT PURSUANT TO 37 CFR 1.132

(LEAVITT DECLARATION)

Sir:

I, Dr. Markley C. Leavitt, hereby declare as follows:

1. I currently hold the position of Vice President of Product Development at AviGenics, Inc, located in Athens, Georgia. I am skilled in the field of the invention. My professional experience and educational background are detailed in the attached Exhibit B.

2. I have reviewed the above-referenced patent application (hereinafter the

“Application”) and I have knowledge of the invention described and claimed in the Application.

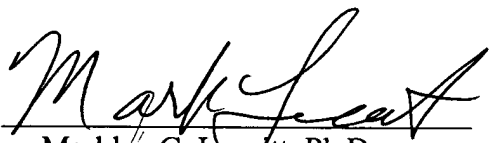
3. I understand that the Examiner rejected claims which are directed to the use of BAC vectors to produce transgenic avians and that the Examiner believes that the patent application does not enable using BAC expression vectors to make transgenic avians and that undue experimentation would be required to use BACs in accordance with the invention.

4. It is my opinion that the Application sufficiently enables a practitioner of skill in the art to produce transgenic avians by injecting BACs into avian embryos and reimplanting the embryos into a recipient hen. Attached Exhibit A shows data for transgenic avians that were produced by the injection of BACs into chicken embryos in accordance with procedure described in the Application. I designed and constructed the BACs used to produce the transgenic avians shown in Exhibit A and oversaw the production of the transgenic avians using the BACs. Production of the BACs was routine from the standpoint that the BACs were operable for producing transgenic avians in accordance with the Application at the time of filing the Application. That is, there is nothing about the BACs that I designed and constructed, which allows them to function successfully in making a transgenic avian, that would not have been apparent to practitioners of skill in the art who had read the Application on or before September 18, 2001.

5. In one particular instance, to produce the BACs, polynucleotide sequences encoding the heavy chain and light chain of an IgG1 (IgG1K) monoclonal antibody were inserted into the 3' UTR of an ovomucoid transcript coding region in two separate BAC vectors. The heavy chain and light chain coding sequences each included a signal sequence located at their 5' ends. For each BAC clone, the coding sequence of each antibody chain and signal sequence was inserted into the ovomucoid coding sequence as an IRES-LC or IRES-HC cassette with the light chain and heavy chain inserts each positioned in the sense orientation. The two BACs were co-injected into chicken embryos to produce the chickens as shown in Exhibit A. Based upon my experience, it is my opinion that the experimentation required to design and construct BACs that can be used for making transgenic avians by injecting BACs into avian embryos and reimplanting the embryos into a recipient hen is routine rather than extensive or undue. In particular, based upon my experience, I

believe that an unmodified BAC (a BAC with no transgene insert) could be used to create a transgenic avian by injecting an unmodified BAC into avian embryo and reimplanting the embryo into a recipient hen.

6. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed 
Markley C. Leavitt, Ph.D.

Dated 6/2/06

Exhibit A

Protein	Constructs injected	DNA Config.	Blood (DNA) Positive/Tested	Semen (DNA) Positive/Tested	Egg White (Protein) Positive/Tested
Recombinant Antibody	Ovomucoid BAC containing antibody Hc and ovomucoid BAC containing antibody Lc coding sequences	Linear	1/103	4/48	17/55

EXHIBIT B

Mark Leavitt, Ph.D.

EDUCATION

Ph.D. (Molecular Biology) 1990, BS (Biology) 1982, University of Arizona, Tucson AZ

PROFESSIONAL EXPERIENCE

2001-present	Principal Scientist to V.P.	AviGenics Inc., Athens, GA
2000-2001	Senior Scientist II	Pfizer Global Research and Development, La Jolla, CA
1994-2000	Scientist to Assoc. Director	Immusol Inc., San Diego, CA
1991-1994	Post-Doctoral Fellow	University of California at San Diego, CA
1985-1990	Research Assistant	University of Arizona, Tucson, AZ

RESEARCH EXPERIENCE

AviGenics, Inc. 2001-present

Biotherapeutics Production in Transgenic Birds

The research group has focused on transgenic expression vector development and transgenic protein characterization. More recently, integration enhancement factors have been utilized to increase transgenesis rates.

- Construction and testing of YAC, BAC, retroviral and plasmid expression vectors.
- Construction and testing of integration enhancement vectors.
- Purification and characterization of transgenic proteins including monoclonal antibodies.
- Production/analysis of transgenic animals.

Pfizer Global Research and Development 2000-2001

Discovery and Development of Antiviral Agents

The group focused on the discovery and development of anti-HIV agents with particular attention to activities against drug-resistant strains.

- Assessment of in vitro antiviral activities of compounds against wild-type and resistant strains.
- Initiation and supervision of external studies.
- Review of virology sections of clinical protocols.
- Generation of research reports and other documents for submission to regulatory agencies.
- Analysis of clinical data related to resistance.

Immusol Inc. 1994-2000

Functional Genomics using Viral Vector Libraries

The group utilized RNA knockout to identify and validate genes relevant to human disease. These studies used ribozyme libraries, randomized in the substrate binding regions, and delivered by retrovirus or recombinant adeno associated virus (rAAV), to engender random gene knockdowns into cell systems.

- Identification of several cellular genes from cDNA libraries which are involved in HCV IRES mediated translation initiation, tumor suppression, Brca-1 expression.
- Design and testing of tetracycline and ecadysone -regulated expression in rAAV and retroviral constructs which contained all control elements within a single vector.
- Design, construction and evaluation of several dozen pol II and III promoters in rAAV and/or retroviral vector backgrounds.

- Initiation of viral vector knockdown collaborations with Pharmaceutical Companies; Millennium, Genzyme and Pfizer.

HIV Gene Therapy/Vector Development

The group developed retroviral vectors and T-cell transduction protocols in support of an investigator initiated IND (Flossie Wong-Staal, UCSD, principal investigator) and a 3 year Pfizer-Immusol collaboration in HIV gene therapy. These efforts culminated in the first clinical trial of ribozyme gene therapy in humans.

- Design, construction and evaluation of multi-operon retroviral and rAAV vectors.
- Generation and assessment of retroviral packaging cell lines.
- Optimization of vector production in serum-free media.
- Completion of pre-clinical studies for an Investigational New Drug (IND) application.
- Management of off-site safety and toxicology studies in support of the IND.
- Preparation and submission of IND application (BB-IND 6405).
- Isolation and *ex vivo* transduction of CD4+ lymphocytes for re-infusion into HIV+ subjects.

University of California at San Diego 1991-1994

Gene Transfer/ HIV Molecular Virology

- Invention of a method for improving the production of rAAV by coupling helper and vector plasmids to adenovirus.
- Development of methods for retroviral transduction of CD4+ lymphocytes and CD34+ stem cells from HIV+ donors.
- Involved in the identification of novel INS elements in the HIV vif gene.
- Novel infectious HIV-2 molecular clone characterized.

University of Arizona 1985-1990

Viral Linear DNA Replication

Studied linear viral DNA replication systems which utilize terminal proteins as primers for DNA synthesis (model for adenoviral and picornaviral replication).

- Identified structural-functional relationships among terminal proteins
- Identified critical conserved residues in viral as well as cellular DNA polymerases.

PUBLICATIONS

1. Leavitt MC, Yu G, Zhou C, and Barber JR (2000) Inhibition of interleukin-1beta (IL-1beta) production in human cells by ribozymes against IL-1beta and IL-1beta converting enzyme (ICE). *Antisense Nucleic Acid Drug Dev.* 10 (6):409-414.
2. Kruger M, Beger C, Li QX, Welch PJ, Tritz R, Leavitt M, Barber JR and Wong-Staal F. (2000) Identification of eIF2Bgamma and eIF2gamma as cofactors of hepatitis C virus internal ribosome entry site-mediated translation using a functional genomics approach. *Proc Natl Acad Sci U S A.* 97(15):8566-8571.
3. Feng Y, Leavitt M, Tritz R, Duarte E, Kang D, Mamounas M, Gilles P, Wong-Staal F, Kennedy S, Merson J, Yu M, and Barber J. (2000) Inhibition of CCR5-dependent HIV-1 infection by hairpin ribozyme gene therapy against CC-chemokine receptor 5. *Viol.* 276: 271-278.
4. Welch PJ, Marcusson EG, Li QX, Beger C, Kruger M, Zhou C, Leavitt M, Wong-Staal F and Barber JR. (2000) Identification and validation of a gene involved in anchorage-independent cell growth control using a library of randomized hairpin ribozymes. *Genomics* 66(3):274-283.
5. Tritz R, Leavitt M and Barber JR (1999) Screening promoter for optimal expression of ribozymes, in *Intracellular Ribozyme Applications: Principles and Protocols*, 115-123, Horizon Scientific Press, Wymondon, U.K.

6. Kraus G, Radaelli A, Talbott R, Leavitt M, Schmidt A, Badel P, Bartz C, Morton W, Wong-Staal F and Looney D. (1998) Characterization of a molecular clone of HIV type 2 infectious for *Macaca nemestrina*. *AIDS Res Hum Retroviruses* 14:65-77.
7. Duarte EA, Leavitt MC, Yamada O, and Yu M. (1997) Hairpin Ribozyme Gene Therapy for AIDS, *Methods in Molecular Biology*, ed. Turner P, p459-468. Humana Press, Totowa, NJ.
8. Welch PJ, Tritz R, Yei S, Leavitt M, Yu M and Barber J. (1996) A potential therapeutic application of hairpin ribozymes: in vitro and in vivo studies of gene therapy for hepatitis C virus infection. *Gene Therapy* 3:994-1001.
9. Leavitt MC, Yu M, Wong-Staal F and Looney D (1996) Ex vivo transduction and expansion of CD4+ lymphocytes from HIV+ donors: prelude to a gene therapy trial. *Gene Therapy* 3:599-606.
10. Mamounas M, Leavitt M, Yu M and Wong-Staal F (1995) Increased titer of recombinant AAV vectors by gene transfer with adenovirus coupled to DNA-polylysine complexes. *Gene Therapy* 2:429-432.
11. Reddy TR, Kraus G, Suhasini M, Leavitt MC and Wong-Staal F (1995) Identification and mapping of inhibitory sequences in the human immunodeficiency virus type 2 vif gene. *J Virol.* 69(8):5167-5170.
12. Yu M, Leavitt MC, Maruyama M, Yamada O, Young D, Ho A and Wong-Staal F (1995) Intracellular immunization of human fetal cord blood stem/progenitor cells with a ribozyme against human immunodeficiency virus type 1. *Proc Natl Acad Sci USA.* 92:699-703.
13. Yu M, Poeschla E, Yamada O, Degrandis P, Leavitt MC, Heusch M, Yee J-K, Wong-Staal F and Hampel A (1995) In vitro and in vivo characterization of a second functional hairpin ribozyme against HIV-1, *Virology* 206:381-386.
14. Yamada O, Kraus G, Leavitt MC, Yu M and Wong-Staal F (1994) Activity and cleavage site specificity of an anti-HIV hairpin ribozyme in human T-cells. *Virology* 205:121-126.
15. Leavitt MC, Yu M, Yamada O, Kraus G, Looney D, Poeschla E and Wong-Staal F (1994) Transfer of an anti-HIV-1 ribozyme gene into primary human lymphocytes. *Human Gene Therapy* 5:1115-1120.
16. Zhu W, Leavitt MC, Jung G and Ito J (1994) Mutagenesis of a highly conserved lysine 340 of the PRD1 DNA polymerase. *Biochim et Biophys Acta.* 1219:260-266.
17. Jung G, Leavitt MC, Schultz M and Ito J (1990) Site-directed mutagenesis of PRD1 DNA polymerase. *Biochem Biophys Res Commun.* 170:1294-1300.
18. Leavitt MC and Ito J (1989) T5 DNA polymerase: Structural-functional relationships to other DNA polymerases. *Proc Natl Acad Sci USA* 86:4465-4469.
19. Jung G, Leavitt MC, Hsieh J-C and Ito J (1987) Bacteriophage PRD1 DNA polymerase: evolution of DNA polymerases. *Proc. Natl. Acad. Sci. USA* 84:8287-8291.
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21. Hsieh J-C, Jung G, Leavitt MC and Ito J (1987) Primary structure of DNA terminal protein of bacteriophage PRD1. *Nucleic Acids Res.* 15:8999-9009.
22. Leavitt MC and Ito J (1987) Nucleotide sequence of Bacillus phage Nf terminal protein gene. *Nucleic Acids Res.* 15:5251-5259.

GRANTS/AWARDS

NIH SBIR Grant "Ovalbumin Locus BACs for Expression of Bio-therapeutics" 2003. Principal Investigator.
NIH SBIR Grant "Use of anti-CCR5 Ribozymes to Inhibit HIV-1 Infection", 1998. Principal Investigator.
DOE Joint Genome Initiative Grant. "Functional Genomics Using Ribozymes" 1998. Co-principal investigator.
NIH Post-Doctoral Fellowship Award, Immunology 1991-1994
Alexander Hollaender Distinguished Postdoctoral Fellowship Award 1991, Department of Energy.